Summary

Galacto-oligosaccharides (GOS) are carbohydrates generated from glucose and galactose generally described by the formula Gal_n-Glc, where n = 2 – 20. GOS exhibit prebiotic functionality which means that they are hardly broken down by human digestive enzymes and therefore reach the intestine almost completely intact. Here, they selectively stimulate the growth of beneficial bacteria.

In the first chapter of this thesis an overview of historical and current literature on the use of β-galactosidases for the synthesis of (galacto-)oligosaccharides was reported. Additionally, a brief description and background of the enzyme (β-galactosidase) and the mechanism for synthesis were provided. Besides an overview of the current literature in the field of GOS synthesis by β-galactosidases, the industrial applications of β-galactosidases were discussed. In industrial applications a need for stable enzymes and the reduction of the production cost exists. Therefore, immobilization of β-galactosidases was further addressed; the possibilities and potential of this technology for oligosaccharide synthesis were discussed. Despite the vast amount of available literature with respect to immobilization and the described potential of these techniques, barriers for the application of this technology still seem to exist. While similar processes have been applied on an industrial scale, such as the hydrolysis of lactose and the production of high fructose corn syrup (HFCS), the application of immobilized enzymes for the production of GOS is still behind.

In Chapter 2, the selection of a suitable carrier for the immobilization and the proposed application in the intended process was described. The selection was carried out

by application of the Analytical Hierarchy Process to evaluate three different carriers. Additionally, the suitability of the AHP was confirmed by comparing its outcome to that of a cost-benefit analysis. Application of AHP methodology on the selection of the most suitable carrier for the immobilization of the β-galactosidase from *B. circulans*, lead to the selection the same carrier as when the a model for the calculation of the cost price per cycle of GOS synthesis was applied. The outcome showed that the AHP was an applicable methodology in decision-making with regard to the selection of a suitable enzyme carrier.

In Chapter 3, the application of immobilized *B. circulans* β-galactosidase in a batch process is described. Particularly, the fact that the conversion of lactose to GOS started from a slurry instead of the more commonly described lactose solution was given attention. The enzymatic and the volumetric productivity of the system were compared with those of a system with free enzyme. In addition, the composition of the final products were compared with the composition of the free enzyme GOS mixture. The immobilized enzyme allowed for shorter reaction times because a higher E/S ratio could be applied. This study showed that the use of immobilized enzyme offers the opportunity to reduce both reaction time and enzyme usage. Moreover, an increase of both enzymatic and volumetric productivity was observed since more GOS was synthesized with the same amount of enzyme. Starting from a lactose slurry contributes to increased sustainability since the preparation of the customary concentrated lactose solution could be omitted and thus saving energy.

Chapter 4 describes the synthesis of GOS by means of immobilized enzyme in a Packed Bed Reactor (PBR). In contrast to the process described in Chapter 3, this

involved a continuous system. The inactivation constants at different temperatures and lactose concentrations were determined. The workable limits of such a continuous process were studied during this work and evaluated. Although increasing substrate concentrations notably increased the stability of the enzyme at 50 °C, the protective properties of a highly concentrated substrate solution could not counteract the effects of thermal deactivation at 60 °C. Despite the increased inactivation of the enzyme at 60 °C, the PBR system could be operated for a substantial amount of time. During this study the limits of operating a packed bed reactor with immobilized β-galactosidase from *B. circulans*, were encountered

In the fifth chapter the formation of allo-lactose during the conversion of lactose to GOS, catalyzed by *B. circulans* β-galactosidase, was described. It was shown that the rate of allo-lactose formation is determined by the enzyme dosage, due to the fact that the reaction is kinetically controlled. The increase of allo-lactose was caused by the (indirect) transglycosylation of galactose to the C-6 carbon of a free glucose molecule and secondly, the hydrolysis of trisaccharides and higher oligosaccharides that were built up from allo-lactose. The direct synthesis of allo-lactose was investigated, however no evidence for this was found. The preferential substrate utilization of β-galactosidase from *B. circulans* gave rise to accumulation of allo-lactose.

In conclusion, this study shows that the use of an immobilized enzyme system can contribute to a more cost-efficient and more sustainable process for the synthesis of GOS. Increased productivity, either in batch- or continuous set up could be achieved while retaining the chemical composition of the galacto-oligosaccharide mixture. Nevertheless,

the challenge for the design of a large scale process starts now. This study provides a good foundation, making it possible to address the challenges of industrial application.